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WJG, 1999 October; 5(5):430-431
World Journal of Gastroenterology
 ISSN 1007-9327

Expression of inducible nitric oxide synthase in human gastric cancer

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Subject headings stomach neoplasms; nitric oxide synthase; nit ric oxide

INTRODUCTION

Inducible nitric oxide synthase (iNOS) is an enzyme that catalyzes the formation of nitric oxide (NO) from L-arginine. iNOS expression and activity results in the production of high levels of NO[1]. The generation of physiological levels of NO is important for mucosal function and it also exerts a cytoprotecti ve effect on the gastrointestinal mucosa. However, increased iNOS expression has been observed in patients with chronic inflammatory diseases of the gastrointes tinal tract, such as ulcerative colitis[2,3], and gastritis[4] and it has been speculated that increased NO may induce DNA damage[5,6] and angiogenesis[7]. Nonetheless, the role of iNOS in human GI neoplasia is largely unkown. Previous studies have demonstrated increased iNOS expression in breast cancer[8,9], and increased iNOS activity and protein levels have been demonstrated in colorectal cancer[10] and adenocarcinoma of the esophagus[11]. However, to date, the role of iNOS in gastric carcinogenesis has not been elucidated.

MATERIALS AND METHODS

Gastric biopsies were obtained from individuals undergoing gastric endoscopy. Two or three mucosal biopsies were endoscopically obtained for histological study. One or two additional biopsies were obtained for mRNA isolation. The biopsies were snap frozen in liquid nitrogen and stored at -80 ℃. The samples used in this study were collected from tumor and a tumor free location in 6 gastric cancer patients, and 7 biopsies were obtained from the histologically normal gastric mucosa in corpus and/or antrum from healthy subjects. RNA was extracted using the RNA-zol B procedure. After completion of this extraction, RNA was separated on a 1.5% agarose gel and RNA was visualized by ethidium bromide staining. cDNAs were generated from one microgram of total RNA; it was denatured at 65 ℃ for 10 min and cooled on ice for 2 min. The RNA was reversely transcribed in a 20 μL final volume of 5x AMV RT buffer, $MgCl_2$, dNTPs, random primers, 16 U of Rnasin and 1.5 U AMV Reverse Transcriptase. The reaction mixture was incubated for 1 hour at 37 ℃, and for 5 min at 96 ℃. For confirmation of cDNA integrity, a RT-PCR analysis using β-actin primers was also performed. The sequence of the primers were as follows: sense primer (s-iNOS), 5' TAGAGGAACATCTG-GCCAGG-3'; antisense primer (as iNOS), 5'-TG-GCAGGGTCCCCTCTGATG-3'; generating a 372 bp fragment of the iNOS transcript. PCR was performed under the following conditions: 94 ℃ for 5 min, 60 ℃ for 45 sec, 72 ℃ for 1min; which was repeated for 35 cycles. Ten mL of the PCR reaction was separated on a 1.5% agarose gel and cDNA was visualized by ethidium bromide staining.

RESULTS

RT-PCR analysis using primers specific for human iNOS mRNA generated a 372 bp fragment of the predicted size. Using this RT-PCR analysis iNOS mRNA was detected in 3 of 6 tumor tissues, and in one of the adjacent tumor free gastric tissues obtained from gastric cancer patients (Table 1). In addition, a fragment of iNOS mRNA was amplified in one of 7 normal gastric tissues obtained from four healthy individuals undergoing endoscopy (Table 2). *H. pylori* infect ion was detected histologically in 5 of 6 cancer patients and in the stomach of two of the four healthy individuals. In two of the *H. pylori* infected indivi duals iNOS mRNA was detected in the non-cancerous mucosa, whereas all individuals without *H. pylori* infection did not exhibit iNOS mRNA.

Table 1 iNOS expression in gastric cancer patients

Patient	Age	Sex	Cancer type	Hp status	iNOS expression	
					Tumor	Tumor-free
1	37	m	Intestinal	+	-	-
2	55	m	Diffuse	+	-	+
3	69	f	Diffuse	+	+	-
4	71	m	Intestinal	+	+	-
5	73	m	Unknown	+	+	-
6	63	m	Intestinal	-	-	-

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Received 1999-08-10

Table 2 iNOS expression in healthy individuals

Patient	Age	Hp	Gastritis	iNOS expression	
				Antrum	Corpus
1	24	-	+	-	-
2	37	+	+	-	+
3	60	+	++	-	nd
4	49	-	-	-	-

DISCUSSION

H. pylori infection of the gastric mucosa may lead to chronic gastritis[12]and to the development of gastric or duodenal ulcers[13]. Furthermore, *H. pylori* infection is considered a risk factor for gastric cancer[14-16]. The molecular alterations underlying the pathogenesis of gastric cancer, however , remain largely unkown. In addition, the molecular alterations induced by *H. pylori* infection of the gastric mucosa which may contribute to gastric carcin ogenesis are not well established. Recently several studies have identified high levels of iNOS expression in *H. pylori* associated gastritis[17,18]. Furthermore, it has been shown that both whole *H. pylori* bacteria and lysates may induce iNOS mRNA levels and iNOS release[11]. Interestingly, after eradication of *H. pylori* infection iNOS expression reverts as determined by immunohistochemistry[18]. In our present study we found that iNOS expression was present only in individuals infected with *H. pylori* infection, whereas individuals without *H. pylori* infection did not exhibit iNOS mRNA in the gastric biopsies.

The chronic inflammation caused by *H. pylori* may induce molecular and cellular pathways contributing to the malignant transformation of the gastric mucosa. In our study 4 of the 6 cancers exhibited iNOS mRNA. While the increased formation of NO may lead to DNA damage, may stimulate angiogenesis, and may inihibit DNA repair mechanisms, the increased expression of iNOS in gastric cancers raisesthe hypothesis that the chronic inflammation caused by *H. pylori* infection may lead to molecular alterations of the gastric mucosa which activate molecular pathways that could lead to the transformation of the gastric mucosa and the development of gastric cancer[19].

In summary, our study supports the hypothesis that molecular alterations induced by *H. pylori* infection of the gastric mucosa may precede the development of gastric cancer and provide a further link between chronic inflammation and malignant transformation in the gastrointestinal tract.

ACKNOWLEDGMENTS This study was supported by a grant from the Land Sachsen-Anhalt (2775A/0087H) awarded to M.P.A. Ebert.

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Edited by MA Jing-Yun